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Susceptibility of Different Embryonic Stages of the Fathead Minnow, Pimephales promelas (rafinnesque), to Linear Alkyl Sulfonate Toxicity

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SUSCEPTIBILITY OF DIFFERENT EMBRYONIC STAGES OF THE FATHEAD MINNOW, PIMEPHALES PROMELAS (RAFINESQUE), TO LINEAR ALKYL SULFONATE TOXICITY

by

Don L. Thompson

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INTRODUCTION

Menner and Dewese (1974) demonstrated that LAS (Linear Aklylbenzene sulfonate), a component of biodegradable determent, is toxic and teratogenic to the embryo Zebra fish (Brachydanic rerio). However, a review of the literature reveals that minimal information is available concerning the effects of LAS on different embryonic stages of the Fathead minnow. The present indepth study has demonstrated that LAS has variable toxicity during embryogenesis of the Fathead minnows.

In commercial preparations of LAS, the length of carbon in the alkyl component varies in a single preparation, from six to sixteen. The compound is designated on the basis of average length of the carbon chain. Two of the widely used surfactants are the 11.2 and 11.8 varieties of LAS (Manner and Dewese, 1974).

A previous study of the effects of twenty pp. 11.2 on the Zebra fish (Brachydanio rerio) by Manner and Thompson, 1974, has continued research into the effects of LAS on the embryogenesis of the Fathead minnow, Pimephales promelas (Rafinesque).

Synthetic detergents have been a controversy of concern for some time. Synthetic detergents began to be produced in large quantities by 1938 (Henderson, 1959), and

started to accumulate in waterways of the United States and Europe by the late 40's and early 50's (Culp and Stoltenburg, 1953; Schmid and Mann, 1961). The existence of these agents raised questions as to the detrimental effects they may have exerted on aquatic life and the quality of drinking water.

By far the largest class of detergents used today are classified as anionic detergents (Davidson and Milwidsky, 1972). Anionic detergents are the most widely used and contribute proportionally more to water pollution problems. These detergents are composed mainly of surfactant and "builder" components. The surfactant is an amphipathic molecule; it has both strong hydrophobic and hydrophibics groups on it. The "builder" is usually a salt that enhances the cleansing action of the detergent.

It is well known that a wide variety of toxic agents are particularly damaging to embryonic life (Wilson, 1973); LAS is such an embryotoxic agent (Pickering, 1966; Hokanson and Smith, 1971).

Fathead minnow was chosen because it is indigenous to America and is frequently used for bioassay by the Environmental Protection Agency. Also, it has been used extensively in toxicity studies (Bender, 1969; Mount and Stephan, 1969; Brungs, 1971; Authur and Eaton, 1971). Most of the results on Fathead minnow embryos are reported either

as per cent of fish hatching, or in terms of the median tolerance limit (TLm) of the embryos with regard to the toxicant being used.

Embryotoxicity studies with aquatic eggs have, for the most part, been dealing with assessing viability or hatchability. These results are usually expressed as the eggs' median tolerance limits (TLm) to a particular surfactant; this TLm value is the concentration at which 50% of the eggs would die after a fixed exposure time (Dourdoroff et al., 1951).

It is the purpose of this paper to determine if there is any stage of embryonic development of Fathead minnow to which 96 hour exposure of LAS is most tocic.

LITERATURE REVIEW

Studies by Manner and Dewese (1974) have demonstrated that 11.2 LAS is toxic and inhibitory to the developing nervous system of Zebra fish embryos at concentrations above ten ppm.

Manner and Dewese (1973) demonstrated that varying concentrations of LAS produce different neural abnormalities in developing Zebra fish. The developing eye was inhibited and although its histology appeared normal, the size was inversely related to the concentration of compound.

Recently it was reported that two of the most widely used species of LAS, i.e. those having hydrocarbon chain lengths averaging 11.2 and 11.8, were teratogenic to the developing nervous systems of embryonic teleosts. Affected were the Zebra fish, Brachydanio rerio (manner and Dewese, 1973). It was also demonstrated by Manner and Thompson (1974) that if the fish embryos were exposed to LAS early in development, such as during the gastrula stages, primarily the central nervous system was damaged. Among the abnormalities observed were decreased brain size, bent neural tubes, deformed otocysts, and uncoordinated movements post-hatch.

It has been shown by Manner and Thompson (1974) that hatching and early post-hatching are also extremely sensitive stages, with 97.2% of the fry dying when treated two days after hatching. The possibility exists that there are two different mechanisms of action of LAS, one occurring during organogenesis, the other during and immediately following hatching. Manner and Muehleman (1975a) substantiated this by indicating that chorion surrounding the Fathead minnow embryo becomes more permeable as embrogenesis progresses. This demonstrates that the chorion becomes linearly more porous to the tritiated uridine as the embryo increases, reaching a maximum at hatching. This has accounted for the possible high degree of susceptibility to toxic aquatic substances at the time of hatching.

LAS is a surface acting agent. This property suggests that it alters the structure of the chorion, thereby changing its permeability; Manner and Muehleman (1974a). This might account for the synergistic reactions reported when LAS was administrated together with DDT (Dugan, 1967) and various other insecticides (Solon et. al. 1969).

Manner and Muehleman (1975b) demonstrated that when Fathead minnow embryos (Pimephales promelas) were subjected to two-hour pulses of 0.25μCi/ml tritiated uridine at five different developmental ages (5, 33, 48, 72, and 96 hours post fertilization), the counts per minute per embryo and

per milligram of embryo increased over the four day period. This reflected an increased permability and uptake by the embryo which dictates that the age of the developing embryos must be considered an important parameter in all aquatic toxicity and teratogenicity testing.

Abel (1974) demonstrated that synthetic detergents are acutely toxic to fish in concentrations between 0.4 and 40 mg/l. Abel indicates that the gill function is most affected by the asphyxiation. Abel observed that low levels of detergents may increase the uptake of other pollutants. Invertebrates, in their juvenile stages, were extremely sensitive to detergents with concentrations below 0.1 mg/l interfering with growth and development in some species.

Dugan and his associates (1964) has reported on the first synergistic toxicity studies with detergents pesticides. Further chronic studies with gold fish (Dugan, 1967) confirmed this work; fish exposed to sublethal levels of LAS for 37 days were extremely sensitive to DDT. A similar synergistic result involving the effects of parathion and LAS were also obtained with Fathead minnow (Solon et. al., 1969). Dugan (1967) proposed that acute toxicities and median tolerance limits did not evaluate the toxicity of pollutants realistically; combined effects of various water contaminants must be investigated.

The mechanisms by which detergents cause their observed effects are not know with certainty (Gouda, 1973). Toxic effects of detergents on living organisms have been studied, largely with regard to bacteriocides. Early research has been reviewed concerning detergent-protein interactions (Putnam, 1948).

The high degree of susceptibility of pregastrular Fathead minnow embryos to LAS (Manner and Thompson, 1974) has prompted the suggestion that the mechanism of action might be related to the genetic processes occurring in the early embryo.

Some investigations have compared the effects of LAS and alkyl benzene sulfonate (ABS). Pickering (1966) found LAS to be more than twice as toxic as ABS when used with Fathead minnow eggs and fry; 9 day TLm values for LAS and ABS were 2.3 and 6.4, respectively. Thatcher and Santer (1967) obtained comparable results with five adult species of fish. The average 96 hour TLm's ranged from 3.3 to 6.4 ppm with LAS and 7.4 to 22.0 ppm with ABS. It was shown by Dooley (1968), that LAS was less toxic than ABS to the mosquito minnow after 72 hours of exposure.

Water hardness is an important factor in the toxicity of may poisons, and the toxicity of detergents has been variously reported to increase, decrease, or be unaffected by increasing water hardnwss. Henderson et. al. (1959)

found ABS to be more toxic to Pimephales promelas (Rafinesque) in hard water than in soft, but a sodium alkyl sulfonate was more toxic in soft water. In contrast, Hokanson and Smith (1971), found water hardness "the most significant environmental variable" in the toxicity of LAS to Sunfish Lempomis macrochirus, mean incipient LC_{50} being given as 4.25 mg/l and 2.85 mg/l in water of hardness 15 mg/l and 290 mg/l as $CaCO_3$m respectively.

Previous studies on detergent biodegradation have been performed using static, dynamic, or field systems. Hammerton (1944) demonstrated the static system in which certain concentrations of detergent surfactant are added to river water and then analyzed for degradation at regular intervals. Swisher (1963z), too, utilized this technique in further demonstrations. The static system is only useful in assessing primary degradation. It will not detect the surfactant if the sulfonate or phenyl portion is removed or if the alkyl group is shortened to fewer than eight cargons (Swisher, 1963a).

The dynamic systems attempt to simulate the conditions of an activated sludge sewage treatment and operate on the principle of continuous feed (Renn et al., 1964: Swisher, 1967). The dynamic systems' biodegradation is generally measured by the ratio of percentage of

surfactant removed to percentage of fermentable organics removed (Renn et al., 1964).

Field tests are the most difficult to do since they require the cooperation of a small community. But they give the most realistic picture of biodegradation rates. Such tests are usually run to confirm the river die-away and/or dynamic methods (Renn et al., 1964).

MATERIALS AND METHODS

Fully grown Fathead Minnows, Pimephales promelas (Rafinesque), were selected for this study because: this fish is frequently used for bioassays by the Environmental Protection Agency; it has been used extensively in toxicity studies.

The following technique was used in breeding Fathead minnows. Inverted halves of 3 inch asbestos pipes were placed on the bottom of an aerated 20 gallon aquarium. These pipes served as egg deposition sites. Two males and eight females were placed into the breeding tank. At approximately 10 a.m. daily, the inverted pipes are removed from the water and examined for the presence of eggs. If found, these eggs were removed by finger or eyedropper and transferred to 10 gallon aeration tank for further treatment. With the finger method the mortality rate of eggs appeared to be considerably less than with the eyedripper method. The eggs were then transferred to a petri dish and maintained in an incubator. The water in the petri dish was changed daily.

Temperature is an important factor in stabilizing the embryonic development of the eggs. Consequently, the eggs were developed at constant temperature of 23 ± 1^{o} C. To ensure that the temperature remained constant at all times,

heaters were placed in the tanks and the thermostats set to provide 23 $\pm$ 1^{o} C. This was necessary in order to avoid temperature fluctuations due to variable room temperature.

The photoperiod was kept at 16 hours light, and 8 hours in dark with a fluorescent light on a timer.

Quietness is essential for breeding. To exclude noise, the door was kept closed at all times except when entering to clean the tanks or to feed the fish. These chores were restricted to people working with the project. The room was equipped with sufficient sound proof material to minimize noise.

The size of adult Fathead minnows range from 50 to 70 mm in length, which size probably is not attained until the second year of age (Scott and Crossman 1973). This appears to be the maximum age of the species Marcus (1934). However, individuals occasionally attained three years of age (Carlson, 1967).

Approximately equal portions of food were fed to the fish twice daily during the course of the experiment. Fathead minnow (Pimephales promelas (Rafinesque) consisted of brine shrimp, trout, chow, and tetramin. The water in the aquarium was changed completely every second week.

Developing empryos of Fathead minnows were exposed to two different commercial preparations of Linear aklyl benzene sulfonate (LAS). One had an average hydrocarbon

chain length of 11.2, while the other had an average of 11.8, as determined by gas chromatograph studies, of the Procter and Gamble Co., Cincinnati, Ohio. The 11.2 compound had an average molecular weight of 234.7 and 73% active surfactant, 5% water and 22% sodium sulfate. The aklyl chain lengths of the sample contained 20.44% C_{10}, 41.48% C_{11}, 31.94% C_{12}, 1.48% C_{13}, and 0.41% C_{14}.

The 11.8 sample was 62% active LAS, 16% water, and 22% sodium sulfate, and had an average molecular weight of 348. The alkyl chain lengths of the preparations were 7.24% C_{10}, 26.16% C_{11}, 55.94% C_{13}, 0.29% C_{14}, with remaining factors being 0.04% less than C_{10} and 0.19% greater than C_{14}.

A total of 3643 Fathead Minnows, Pimephales promelas (Rafinesque), were used in this study. These eggs were collected and placed into their respective solutions at a density of ten per petri dish. One hundred eggs/day during days 0-7 of development were treated for 24 hours with a concentration of 20 ppm of 11.2 and 11.8 LAS. The onset of the period of exposure differed for each group. Controls were assayed at 0, 24, 48, 72, 96, 120, 144, 168, 192, 216, and 240 hours after fertilization. After 24 hour exposure, the viable embryos were counted.

Concentrations used in the experimental studies were 2.5, 5, 7, 9, 12, and 20 ppm of 11.2 and 11.8 LAS.

The solutions were made daily and changed for the duration of the test. Embryos were scored daily for deaths.

Criteria for Determining a Viable Embryo of the Fathead Minnow

Viable embryos were determined by observation under a microscope. If the embryo appeared a milky white color, the embryo were not fertile. However, if the embryo appeared clear in coloration the embryo was then fertile.

Statistical Analysis

TLm values were obtained as suggested by Sprague (1969), with the time of death being observed at the end of 96 hours. The TLm 96 hours exposures to 11.2 or 11.8 LAS were a function of day of treatment.

RESULTS

Each group of Fathead minnows was observed for a period of four days. At the end of this period, the number of dead fish was determined percentage-wise.

The data illustrated that the embryos became more susceptible to 11.2 LAS concentration as they became older. With the 11.8 LAS concentrations, there is no significant change between Day 0 and Day 7.

TLm Values

To determine TLm graphs, actual points were plotted and a best fit line determined. These graphs are pictured in Figures One and Two.

Further Observations

All the Fathead minnow embryos subjected to 7, 9, 12, and 20 ppm of 11.8 LAS were dead at Day 4. It was demonstrated by Manner and Dewese (1973) that all Zebra fish embryos subjected to 10, 15, and 20 ppm of 11.8 LAS were dead within 24 hours. The embryos at these concentrations were all abnormal after three hours of exposure to detergent. The 11.8 LAS at 5 and 2.5 ppm embryos subjected either were dead or deformed.

DATA - 11.2 AND 11.8 LAS

CONTROL

Two sets of control were used during the experiment. The controls were not subjected to the surfactants (11.2 and 11.8 LAS respectively). The embryos were placed in fresh water for a period of 96 hours. At the end of the 96 hour period, the percentage of dead embryos was determined. In one set of controls (11.2 LAS) using 100 embryos, at the end of 96 hours, there were 2 dead, having a percentage of 2%. In the other control (11.8 LAS) using 109 embryos, at the end of 96 hours there were 0 deaths, having a percentage of 0%. Therefore, no significant percentage of deaths occurring in the 96 hour period.

Table 1 - 16: The data shown in 11.2 and 11.8 LAS, respectively, illustrate actual and theoretical data. Actual data illustrates:

X = ppm

Y = % dead at 96 hours after treatment. Percentage of dead embryos subjected at respective days to the respective concentrations of 11.2 and 11.8 LAS for each day of treatment.

Theoretical data illustrates the linear regression line TLm 96; (linear regression is descriptive term and indicates

that all the population means being considered are on a straight line).

Table 17 - 22: The data show TLm 96 for 11.2 and 11.8 LAS respectively. Illustrating the respective concentration ppm of LAS (2.5, 5, 7, 9, 12, and 20), the number of embryos to start; the number of dead embryos at 96 hours and the number of alive embryos at 96 hours.

TABLE ONE

Data - 11.2 LAS

DAY 0

x	20	12	9	7.9	5.0	2.5	Actual
y	56	10	18	30	0	20	

x	40	30	20	10	Theoretical
y	92.04	69.26	46.48	23.70	TLm 96 = 21.6

DAY 1

x	20	12	9	7.9	5.0	2.5	Actual
y	68	20	33	0	0	50	

x	40	30	20	10	Theoretical
y	95.8	73.81	51.81	29.82	TLm 96 = 19.2

DAY 2

x	20	12	9	7.9	5.0	2.5	Actual
y	80	10	3	0	0	10	

x	40	30	20	10	Theoretical
y	147	105	62	19.73	TLm 96 = 17.2

DAY 3

x	20	12	9	7.9	5.0	2.5	Actual
y	91	6	0	10	30	0	

x	40	30	20	10	Theoretical
y	158	113	69.69	25.49	TLm 96 = 15.6

DAY 4

x	20	12	9	7.9	5.0	2.5	Actual
y	88	3	0	10	0	0	

x	40	30	20	10	Theoretical
y	195	135	75.38	15.53	TLm 96 = 17.41

DAY 5

x	20	12	9	7.9	5.0	2.5	Actual
y	68	20	0	0	0	0	

x	40	30	20	10	Theoretical
y	191	129	68.25	6.80	TLm 96 = 17.0

DAY 6

x	20	12	9	7.9	5.0	2.5	Actual
y	94	70	9	0	0	0	

x	40	30	20	16	8	Theoretical
y	255	179	102	72.15	10.90	TLm 96 = 13.0

DAY 7

x	29	12	9	7.9	5.0	2.5	Actual
y	100	0	0	0	0	0	

x	40	30	20	10	Theoretical
y	100	100	100	0	TLM 96 = 10

TABLE 2

Data - 11.8 LAS

DAY 0	x / 20 / 12 / 9 / 7.9 / 5.0 / 2.5 /	Actual
	y/ 100 / 76 / 33 / 70 / 0 / 10 /	
	x / 40 / 30 / 20 / 15 / 10 / 5 /	Theoretical
	y/ 221 / 165 / 108 / 79.97/ 51.57/23.18/	TLm 96 = 9.8
DAY 1	x / 20 / 12 / 9 / 7.9 / 5.0 / 2.5 /	Actual
	y/ 0 / 80 / 39 / 70 / 20 / 60 /	
	x / 20 / 15 / 10 / 5 /	Theoretical
	y/ 20.37/ 31.28/ 42.19/ 53.10/	TLm 96 = 6.4
DAY 2	x / 20 / 12 / 9 / 7.9 / 5.0 / 2.5 /	Actual
	y/ 1 / 100 / 100 / 0 / 0 / 0 /	
	x / 20 / 15 / 10 / 5 /	Theoretical
	y/ 20.99 / 39.81 / 58.62 / 77.44 /	TLm 96 = 12.2
DAY 3	x / 20 / 12 / 9 / 7.9 / 5.0 / 2.5 /	Actual
	y/ 100 / 100 / 90 / 60 / 0 / 17 /	
	x / 20 / 15 / 10 / 5 /	Theoretical
	y / 191 / 137 / 82.93 / 28.64 /	TLm 96 = 7.0

DAY 4

x	20	12	9	7.9	5.0	2.5	Actual
y	100	100	60	100	0	0	

x	20	15	10	5	Theoretical
y	100	100	84.83	19.81	TLm 96 = 7.4

DAY 5

x	20	12	9	7.9	5.0	2.5	Actual
y	100	100	80	0	0	0	

x	20	15	10	7	5	Theoretical
y	100	100	67.57	5.62	0	TLm 96 = 9.2

DAY 6

x	20	12	9	7.9	5.0	2.5	Actual
y	100	100	80	0	0	40	

x	20	15	10	5	Theoretical
y	100	100	65.21	26.22	TLm 96 = 8.1

DAY 7

x	20	12	9	7.9	5.0	2.5	Actual
y	100	100	100	10	10	0	

x	20	15	10	5	Theoretical
y	100	100	75.8	17.35	TLm 96 = 7.8

TLm 96

DATA

TLm 96

11.2 LAS

2.5ppm	Start	Dead	Alive
Day 0	15	3	12
Day 1	10	5	15
Day 2	10	1	9
Day 3	10	0	10
Day 4	10	0	10
Day 5	10	0	10
Day 6	10	0	10
Day 7	10	0	10

11.2 LAS

5ppm	Start	Dead	Alive
Day 0	10	0	10
Day 1	10	0	10
Day 2	11	0	11
Day 3	10	3	7
Day 4	10	0	10
Day 5	10	0	10
Day 6	10	0	10
Day 7	10	0	10

11.2 LAS

7.94ppm	Start	Dead	Alive
Day 0	10	3	7
Day 1	10	0	10
Day 2	10	0	10
Day 3	10	1	9
Day 4	10	1	9
Day 5	10	0	10
Day 6	10	0	10
Day 7	10	0	10

TLm 96

11.2 LAS

7.94ppm	Start	Dead	Alive
Day 0	10	3	7
Day 1	10	0	10
Day 2	10	0	10
Day 3	10	1	9
Day 4	10	1	9
Day 5	10	0	10
Day 6	10	0	10
Day 7	10	0	10

11.2 LAS

9ppm	Start	Dead	Alive
Day 0	110	20	90
Day 1	100	33	67
Day 2	10	3	7
Day 3	10	0	10
Day 4	10	0	10
Day 5	10	0	10
Day 6	11	1	10
Day 7	10	0	10

11.2 LAS

12ppm	Start	Dead	Alive
Day 0	100	10	90
Day 1	10	2	8
Day 2	10	1	9
Day 3	70	8	62
Day 4	77	2	75
Day 5	10	2	8
Day 6	10	7	3
Day 7	10	0	10

TLm 96

11.2 LAS

20ppm	Start	Dead	Alive
Day 0	146	82	64
Day 1	103	70	33
Day 2	99	81	18
Day 3	102	93	9
Day 4	126	111	15
Day 5	78	53	25
Day 6	132	124	8
Day 7	89	89	0
Day 8	108	108	0

11.8 LAS

2.5ppm	Start	Dead	Alive
Day 0	10	1	9
Day 1	10	6	4
Day 2	11	0	11
Day 3	12	2	10
Day 4	10	0	10
Day 5	10	0	10
Day 6	10	4	6
Day 7	10	0	10

11.8

5ppm	Start	Dead	Alive
Day 0	10	0	10
Day 1	10	2	8
Day 2	10	0	10
Day 3	10	0	10
Day 4	10	0	10
Day 5	10	0	10
Day 6	10	0	10
Day 7	10	1	9

TLm 96

11.8 LAS

7.94ppm	Start	Dead	Alive
Day 0	10	7	3
Day 1	10	7	3
Day 2	11	0	11
Day 3	10	6	4
Day 4	10	10	0
Day 5	10	0	10
Day 6	10	0	10
Day 7	10	1	9

11.8 LAS

9ppm	Start	Dead	Alive
Day 0	100	33	67
Day 1	100	31	69
Day 2	11	11	0
Day 3	10	9	1
Day 4	10	6	4
Day 5	10	8	2
Day 6	10	8	2
Day 7	10	10	2

11.8 LAS

12ppm	Start	Dead	Alive
Day 0	100	10	90
Day 1	10	2	8
Day 2	10	1	9
Day 3	70	8	62
Day 4	77	2	75
Day 5	10	2	8
Day 6	10	7	3
Day 7	10	0	10

TLm 96

11.8 LAS

20ppm	Start	Dead	Alive
Day 0	154	154	0
Day 1	93	0	93
Day 2	97	1	96
Day 3	56	56	0
Day 4	70	70	0
Day 5	62	62	0
Day 6	100	100	0
Day 7	123	123	0

Total = 3643 embryos
TLm 96 = Concentration which kills 50%
4 days after treatment

Figure One 11.8 LAS

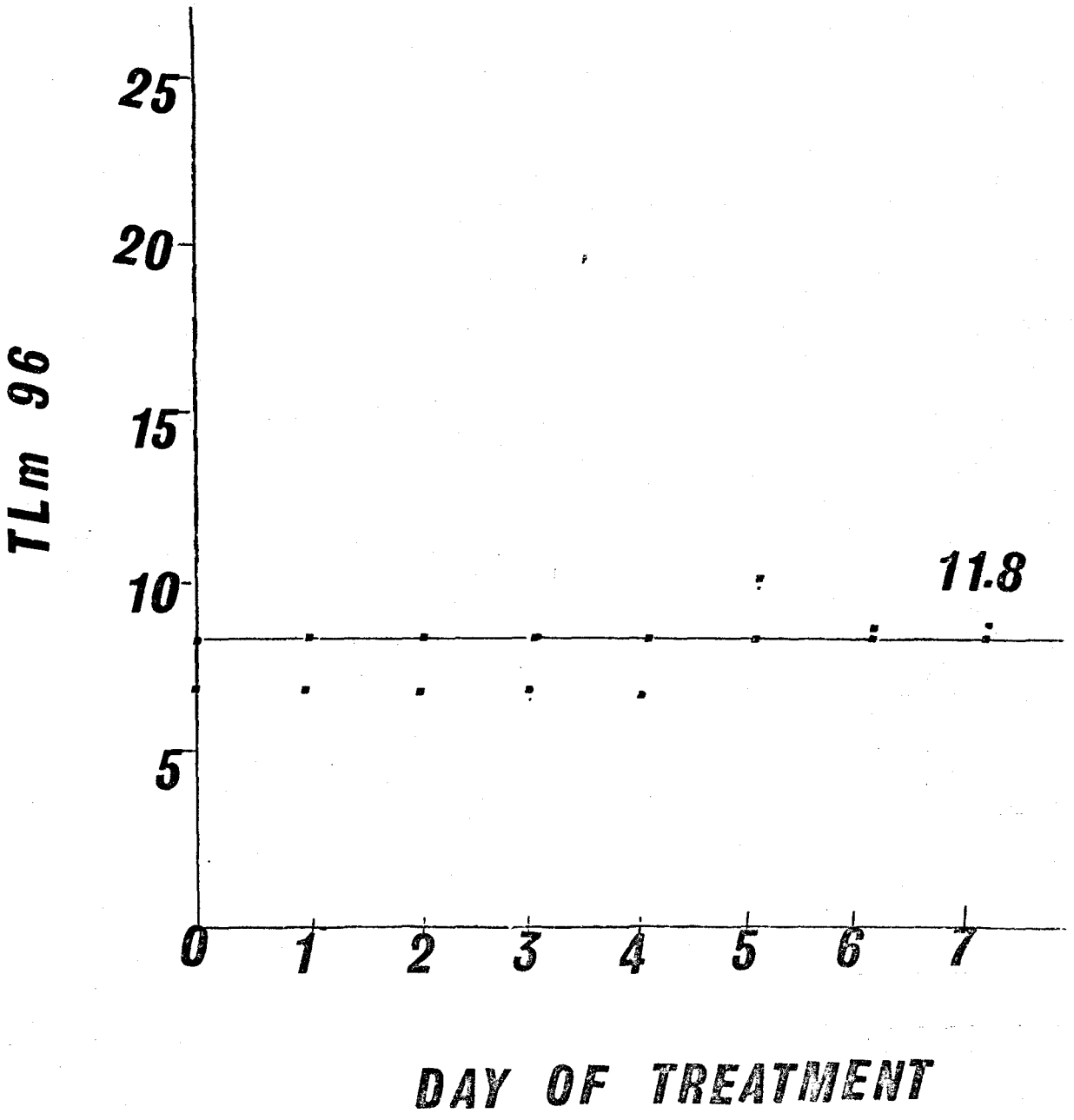
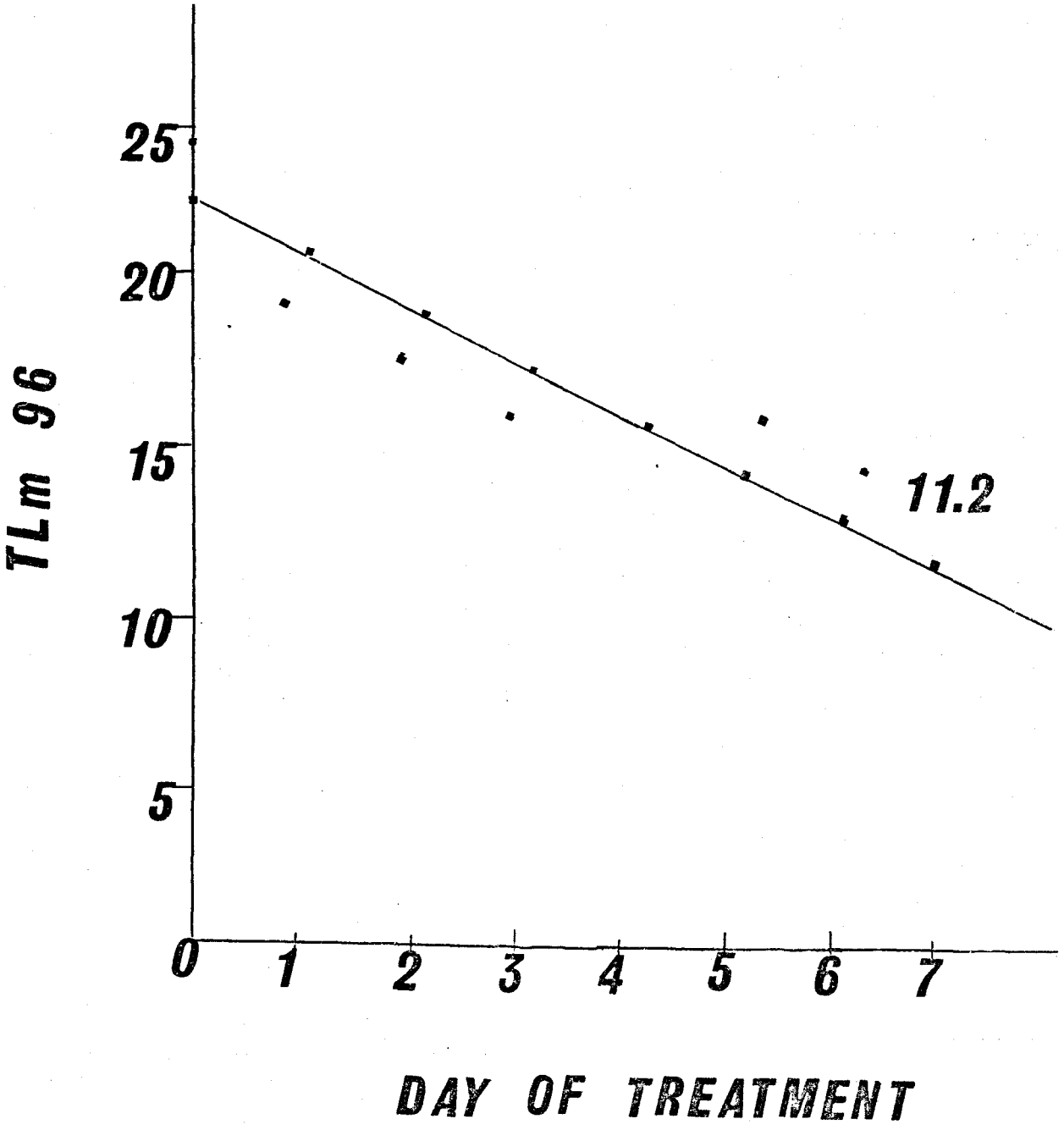

TLm 96
25
20
15
10
5
11.8
0
1
2
3
4
5
6
7
DAY OF TREATMENT

Figure Two 11.2 LAS


TLm 96
25
20
15
10
5
0
1
2
3
4
5
6
7
11.2
DAY OF TREATMENT

DISCUSSION

Most biodegradable detergents are composed primarily of the compound, sodium-n-dodecyl benzene sulfonate (LAS). The linear alklbenzene sulfonates (LAS) are presently the most prominent group of biodegradable surfactant produced by the detergent industry (Harris et al.,) 1971; Davidson and Milsidsky, 1972). Manner and Dewese (1973) in the preliminary study demonstrated that this substance produced several abnormalities in developing Zebra fish.

Manner and Thompson (1974) confirmed that LAS was toxic and inhibitory to the developing nervous system of embryos of the Zebra fish. Branchydanio rerio, at concentrations of 10 ppm and above. It was further demonstrated that the earliest stage of maximum susceptibility to 20 ppm LAS 11.2 occurred during the first two days, when organogenesis was occurring during this time; the mortality rate reached 77.5%; this period was followed by a growth phase during which susceptibility decreased, with the death rate dropping to 55%. Hatching and early post-hatching were also extremely sensitive stages, with 97.3% of the embryos dying when treated two days after hatching. The possibility exists that there are two different mechanisms of action of LAS; one occurring during organogenesis, the other

during and immediately following hatching.

In this present investigation there was a consistent linear regression line using 11.8 LAS for the 20 ppm on the Fathead minnow fish. The earliest stage of maximum susceptibility occurred during days 1 to 7; the mortality rate was 91.8%. This result correlates with the work of Manner and Thompson(1974) in which there was no difference in maximum susceptibility (mortality).

The lowest non-toxic dose of LAS for each of the four day periods of 11.2 and 11.8 LAS was 2.5 ppm. At this dose, less surfactant penetrated the membrane, minimized the effect on the embryo. There was more mobility using the 11.2 LAS after post-hatching. At this dose level, the post-hatching rate of survival was comparable to that of controls.

It is conceivable that a possible cause of why 11.8 LAS (day 1 and 2 embryo), and 11.2 LAS embryo (day 5), mortality rate is slighter than at other stages of development. This indicates that detergents do enter the cell membrane and cause side effects and possibly death. However, this rate of surfactant at entering the embryos does not necessarily destroy the morphologic development.

A possible cause of the slight mortality rate of day 1-2 embryos and day 5 embryos is the sensitivity of the

membrane to the surfactant. Exhibiting a chemical effect on the membrane which digests part of the surfactant and is localized in possibly some area of the membrane until further stages no longer can hold the surfactant. Day 5 is at the post-hatching stage with the membrane in a process of erupting; consequently, the surfactant is engulfed by the fry causing possible internal and other side effects resulting in mortality. Day 1 and 2 embryos are still at embryonic stages.

Manner and Hardwick (1975) demonstrated that as time passes the membrane becomes more resistant to the surfactant. This may be reflected in that in certain stages susceptability to LAS is more prevalent than in other stages. An equally promising theory is that LAS may effect the nucleotide acid soluble pool. It may reflect a difference in RNA being labeled. Work by Manner and Hardwick (personal communication) has shown that RNA of LAS treated embryos do show a difference from that of the normal. Whether or not this is a reflection of precurser pool side or direct gene turn-on is apt to be elucidated.

From the studies of the investigation another possible cause of mortality rate of day 1-7 embryos 11.8 LAS as opposed to those subjected to 11.2 LAS may be related to the shift in alkyl chain lengths.

This study has indicated differences in toxicity

between the 11.2 and 11.8 LAS which may be related to the shift in alkyl chain lengths. 65-28% of the 11.8 LAS alkaylate lengths are 12 carbons or longer while only 33.83% of the 11.2 LAS have similar lengths. Swisher (1964) saw a relationship between toxicity and alkyl chain length. They worked with C_{12} and C_{14} homologs and found the 24 hour TLm's to be 3.1 and 0.64 respectively. It is not determined why the longer alkyl lengths would be more toxic. The degradation intermediates of LAS are not all known as yet even though Swisher (1967) and Michael (1968) have characterized some. It may be possible that longer intermediates prove to be more toxic to aquatic organisms. It is known that the best detergency in LAS is obtained with an average hydrocarbon chain length of 12.5 (Sweeney and Olson, 1964). Since detergency is related to surfactant properties, it is conceivable that the surfactant closer to 12.5 alkylate length would be more effective as a surface-active agent.

Surfactants comprise only a small part of the pollutions found in waterways. Other contaminants may be acted upon by even lower concentrations of surfactants. For example, it is known that surfact-active agents are able to solubilize polycyclic hydrocarbons (known teratogenic agents) that result from industrial and agricultural pollution of waterways. It is believed that fish in such

areas contain high concentrations of such carcinogens (Elworth, 1968). Further tests are necessary to assess the effects of chronic exposure of Zebra fish embryos and larvae to LAS. These acute toxicity studies do, however, indicate that LAS is a teratogenic agent to developing Zebra fish if they are exposed to it at the high blastula stage(which precedes gastrulation by 2 hours). It is known that gastrulation is the most critical stage in the development of Zebra fish (Weis, 1968). It is evident that the 11.8 LAS concentrations of 10 ppm and higher disrupt the embryo at its critical period.

SUMMARY

Linear Alkyl Sulfonate toxicity has been considered a much more "biologically safe" detergent by some scientists because of its faster degradation rate (Swisher, 1963a; Tarring, 1965); it is thought that its toxic potential should be much lower since its primary degradation is accomplished so much more rapidly than that of ABS. However, even low concentrations of detergent surfactant have been reported to be teratogenic (Bergel, 1974) and toxic, depending on the developmental stage and species.

In this study embryos of Fathead Minnow, Pimephales promelas (Rafinesque), were subjected to varying concentrations of 11.2 and 11.8 LAS preparations. The 11.8 LAS was found to be the more toxic of the two.

It could be concluded that Linear Alkyl Sulfonate (LAS) becomes more toxic as embryo ages; some embryos are extremely susceptible to LAS and die soon after exposure; other embryos are extremely resistant and survive (greater than 50%) exposure to LAS for long periods.

APPENDIX

The method for making up solutions for 11.2 LAS was: 27.38 mg of the 11.2 preparations were added to 100 ml of dechlorinated water to make a stock "solution." The stock "solution" was thoroughly mixed with a magnetic stirrer and the following dilutions made:

20 ml of stock to 253 ml aged aquarium water = 20 pp.

12 ml of stock to 261 ml aged aquarium water = 12 ppm

9 ml of stock to 264 ml aged aquarium water - 9 ppm

5 ml of stock to 268 ml aged aquarium water = 5 ppm

2.5 ml of stock to 270.5 ml aged aquarium water = 2.5ppm

11.8 LAS concentrations were made up in a similar way except that 32.26 mg were weighed out since this preparation was 62% active LAS.

Formula used to calculate theoretical TLm 96 (pp 17-20).

$$y = a_0 + a_1 x$$

$$a_1 = \frac{\Sigma x_1 y_1 - \frac{\Sigma x_1 \; \Sigma y_1}{n}}{\Sigma x_1^2 - \frac{(\Sigma x_1)^2}{n}}$$

$$a_0 = \bar{y} - a_1 \bar{x}$$

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APPROVAL SHEET

The thesis/dissertation submitted by DON THOMPSON
has been read and approved by members of the Department of BIOLOGY.

The final copies have been examined by the director of the thesis/dissertation and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the thesis/dissertation is now given final approval with reference to content and form.

The thesis/dissertation is therefore accepted in partial fulfillment of the requirements for the degree of Master of Science.

Oct. 9, 1978
DATE

Harold W. Manner
ADVISOR'S SIGNATURE